Practitioner's Docket No. 56212 (71526) *PATENT*

TRANSMITTAL LETTER TO THE U.S. DESIGNATED OFFICE (DO/US)-- ENTRY INTO THE U.S. NATIONAL STAGE UNDER CHAPTER I

PCT/JP00/07992	13 November 2000	16 November 1999
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

DEVELOPMENT OF METHOD FOR SCREENING PHYSIOLOGICALLY ACTIVE PYRROLE IMIDAZOLE DERIVATIVE
TITLE OF INVENTION

Hiroshi SUGIYAMA, Isao SAITO, Hirokazu IIDA
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: DO/US

NOTE: The completion of those fling requirements that can be made at a time later than 20 months from the priority date results from the Commissioner exercising his judgment under the authority granted under 35 U.S.C. § 371(d). The filing receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 C.F.R. § 1.491, which states: "An international application enters the national stage when the applicant has filed the documents and fees required by 35 U.S.C. § 371(c) within the periods set forth in § 1.494 and § 1.495."

WARNING: *Where the items are those that can be submitted to complete the entry of the international application into the national phase subsequent to 20 months from the priority date, the application is still considered to be in the international stage. And if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (because international application papers are not covered by an ordinary certificate of mailing. 37 C.F.R. § 1.8(2)(xi)).*

CERTIFICATION UNDER 37 C.F.R. § 1.10*
*(Express Mail label number is **mandatory.**)*
(Express Mail certification is optional.)

I hereby certify that this paper, along with any document referred to, is being deposited with the United States Postal Service on this date July 16, 2001, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number **EL895418445US**, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Susan M. Dillon
(type or print name of person mailing paper)

Susan M Dillon
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

WARNING: *Documents and fees must be clearly identified as a submission to enter the national stage under 35 U.S.C. § 371, otherwise the submission win be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).*

WARNING: *Failure to pay the national fee within 20 months from the priority date will result in the abandonment of the application. The time for payment of the basic fee is not extendable. M.P.E.P. § 1893.01(a)(1), 6th ed., rev. 3.*

1. Applicant herewith submits to the United States Designated Office (DO/US) the following items under 35 U.S.C. 371:

a. [X] This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. [X] The U.S. National Fee (35 U.S.C. § 371(c)(1)) and
[X] other fees (37 C.F.R. § 1.492), as indicated below:

2. Fees

CLAIMS FEE	**(1) FOR**	**(2) NUMBER FILED**	**(3) NUMBER EXTRA**	**(4) RATE**	**(5) CALCULATIONS**
*	**TOTAL CLAIMS**	**24 - 20 =**	**4**	**x$ 18.00=**	**$72.00**
	INDEPENDENT CLAIMS	**4 - 3 =**	**0**	**x$ 80.00=**	**$0**
	MULTIPLE DEPENDENT CLAIMS(S) (if applicable) + $270.00				**$270.00**
BASIC FEE**	**The international search fee, as set forth in § 1.445(a)(2) to be paid to the US PTO acting as an international Searching Authority:** **[ ] has been paid (37 CFR 1.492(a)(2))..... $760.00** **[ ] has not been paid (37 CFR 1.492(a)(3))..... $970.00** **[X] where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) $860.00**				**$ 860.00**
	Total of above Calculations				**= $1,202.00**
SMALL ENTITY	**Reduction by ½ for filing by small entity, if applicable. Affidavit must be filed also. (note 37 CFR 1.9, 1.27, 1.28)**				**-**
	Subtotal				**$1,202.00**
	Total National Fee				**$1,202.00**
	Fee for recording the enclosed assignment document $40.00 (37 CFR 1.21(h)). (See Item 10 below). See attached "ASSIGNMENT COVER SHEET (37 CFR 3.34)".				**$ 40.00**
TOTAL	**Total Fees enclosed**				**$1,242.00**

***WARNING:* *"To avoid abandonment of the application, the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 20 months from the priority date; *** (2) the basic national fee (see § 1.492(a)). The 20-month time limit may not be extended." 37 C.F.R. § 1.494(b).*

i [X] A check in the amount of $ 1,242.00 to cover the above fees is enclosed.
ii. [ ] Please charge Account No. _________ in the amount of $ ______________.
A duplicate copy of this sheet is enclosed.

WARNING: *If the translations of the international application and/or oath or declaration have not been submitted by the applicant within twenty (20) months from the priority date, the applicant will be so notified and given a period of time within which to file the translation and/or oath or declaration in order to prevent abandonment. The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than twenty (20) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than twenty (20) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 will apply. 37 C.F.R. § 1.494(c).*

3. A copy of the International application as filed (35 U.S.C. § 371(c)(2)):
   a. [ ] is transmitted herewith.
   b. [ ] is not required, as the application was filed with the United States Receiving Office.
   c. [X] has been transmitted
      i. [X] by the International Bureau. Date of mailing of the application Prom form PCT/IB/308): **25 May 2001**.
      ii. [ ] by applicant on _____________.
      Date

NOTE: Section 1.494(b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 20 months from the priority date to avoid abandonment. "The International Bureau nominally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies the applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage and applicant has received notice from the International Bureau, applicant need only pay the basic national fee by 20 months from the priority date." [This can now be paid subsequently with a surcharge.] Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35.

4. A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):
   a. [X] is transmitted herewith.
   b. [ ] is not required as the application was filed in English.
   c. [ ] was previously transmitted by applicant on _____________.
   Date

5. [X] Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: The Notice of January 7, 1993 indicates that 37 C.F.R. § 1.494(d) was "amended to clarify the existing practice that PCT Article 19 Amendments must be submitted by 20 months from the priority date, which time may not be extended." This Notice further advises: "Of course, the failure to do so does not result in loss of the subject matter of PCT Article 19 amendments. The applicant may submit that subject matter in a preliminary amendment filed under Section 1.121. In many cases, filing an amendment under Section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 35. See item 11(c) below. See also 37 C.F.R. § 1.494(d).
   a. [ ] are transmitted herewith.

b. [ ] have been transmitted
   i. [ ] by the International Bureau. Date of mailing of the amendment (from form PCT/IB/308): ____________.
   ii. [ ] by applicant on __________________________.
   Date

c. [X] have not been transmitted, as
   i. [ ] no notification has been received that the International Search Authority has received the Search Copy.
   ii [ ] the Search Copy was received by the International Searching Authority, but the Search Report has not yet been issued. Date of receipt of Search Copy prom form PCT/ISA/202):______________.
   iii. [X] applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210): **13 February 2001**
   iv. [ ] the time limit for the submission of amendments has not yet expired. The amendments, or a statement that amendments have not been made, will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)):
   a. [ ] is transmitted herewith.
   b. [ ] is not required as the amendments were made in the English language.
   c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] An oath or declaration of the inventor including power of attorney (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115
   a. [ ] was previously submitted by applicant on ______________
   Date
   b. [X] is submitted herewith, and such oath or declaration
      i. [X] is attached to the application.
      ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or (c) and 5(b); and states that they were reviewed by the inventor, as required by 37 C.F.R. § 1.70.
      iii. [ ] will follow.

II. Other document(s) or information included:

8. [X] An international Search Report or Declaration under PCT Article 17(2)(a):
   a. [ ] is transmitted herewith.
   b. [X] has been transmitted by the International Bureau. Date of mailing from form PCT/IB/308): **25 May 2001**.
   c. [ ] is not required, as the application was searched by the United States International Searching Authority.
   d. [ ] will be transmitted promptly upon request.
   e. [ ] has been submitted by applicant on _______________.

Date

f. [ ] is not transmitted, as the international search has not yet issued.

9. [X] An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:
   a. [ ] is transmitted herewith.
      Also transmitted herewith is (are)
      [ ] Form PTO-1449 (PTO/SB/08A and 08B)
      [ ] Copies of citations listed
   b. [X] will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).
   c. [ ] was previously submitted by applicant on _____.
      Date

10. [X] An assignment document is transmitted herewith for recording. A separate
    [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or
    [X] FORM PTO—1595 is also attached.
    [X] Please mail the recorded assignment document to:
       i. [X] the person whose signature and address appears below.
       ii. [ ] the following:

11. [X] Additional documents
    a. [ ] Copy of request (PCT/RO/101)
    b. [X] International Publication No. WO 01/36677
       i. [X] Specification, claims and drawing
       ii. [ ] Front page only
    c. [ ] Preliminary amendment (37 C.F.R. § 1.121)
    d. [X] Other: Form PCT/ISA/210
       Form PCT/IB/304
       Form PCT/IB/308
       Form PCT/RO/105
       Form PCT/IB/301

12. [X] The above checked items are being transmitted
    a. [ ] before the 18th month publication.
    b. [X] after publication and the article 20 communication, but before 20 months from the priority date.
    c. [ ] after 20 months (revival).

NOTE: Petition to revive (37 C.F.R. § 1.137(a) or (b)) is necessary if 35 U.S.C. § 371 requirements are submitted after 20 months.

13. [ ] Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____ namely:
    Date

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: *"A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).*

NOTE: *"Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).*

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 04-1105.

[X] 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 20 months without extension (37 C.F.R. § 1.494(b)(2)), results in abandonment of the application, it would be best to always check the above box.*

[X] 37 C.F.R. § 1.492(b), (c), and (d) (presentation of extra claims)

NOTE: *Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment, prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.*

[X] 37 C.F.R. § 1.17 (application processing fees)
[X] 37 C.F.R. § 1.17(a)(1)-(5)(extension fees pursuant to § 1.136(a).

[ ] 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b)).

NOTE: *Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).*

NOTE: *37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying or at the time of paying . . . issue fee...." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.*

[ ] 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 20 months after the priority date.

Reg. No. 33,860

SIGNATURE OF PRACTITIONER

Peter F. Corless
(type or print name of practitioner)

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PCT/US00/00359

Practitioner's Docket No. 56212 (71526) *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	H. Sugiyama et al.		
Application No.:	09/889,379	Group No.:	Not Yet Assigned
Filed:	July 16, 2001	Examiner:	Not Yet Assigned
For:	DEVELOPMENT OF METHOD FOR SCREENING PHYSIOLOGICALLY ACTIVE PYRROLE IMADOLE DERIVATIVE		

Assistant Commissioner for Patents
Washington, D.C. 20231
BOX: PCT

SIR:

STATEMENTS IN SUPPORT OF FILING AND

SUBMISSIONS IN ACCORDANCE WITH 37 CFR §§1.821 - 1.825

In accordance with 37 CFR §§1.821 - 1.825, I hereby state that the content of the paper, computer-readable copies of the sequence listing submitted in accordance with 37 CFR §1.821(c) and (e), respectively, are the same.

Respectfully submitted,

Peter F. Corless (Reg. 33,860)
EDWARDS & ANGELL, LLP
P.O. Box 9169
Boston, MA 02109
(617) 439-4444

Date: March 11, 2002

Docket No. 56212 (71526)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: H. Sugiyama et al.

Serial No.: 09/889,379

Filed: July 16, 2001

For: Development of Method for Screening Physiologically Active Pyrrole Imidazole Derivative

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, DC 20231

Sir:

AMENDMENT

Please amend the application as follows.

IN THE SPECIFICATION:

On page 5, line 16, under the heading "Brief Description of the Drawings" and after "and DNA", please add --(SEQ ID NOS:1-2).--.

On page 5, line 18, under the heading "Brief Description of the Drawings" and after "in Fig. 1", please add --(SEQ ID NOS:3-4)--.

REMARKS

Applicants have amended the specification to comply with the "Notification to Comply with Requirements". The amendments are solely to identify the amino acid sequences referred to in the specification with the sequence listing ID numbers. No new matter is added by virtue of these amendments.

Applicants submit herewith the Sequence Listing (1 page) to include the Sequence Listing as part of this Application.

Further enclosed is a computer readable copy of the above-mentioned copy of the Sequence Listing. That copy is the same as the copy of the Sequence listing.

The Commissioner is hereby authorized to charge any fees which may be required to consider this submission to Deposit Account No. **04-1105.**

Early examination and allowance of the application are respectfully requested.

Respectfully submitted,

Peter F. Corless
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

On page 5, line 16:

--Fig. 1 is a photograph instead of a drawing which shows a result of reaction with ImPyLDu86 of the present invention and DNA (SEQ ID NOS:1-2).--

On page 5, line 18:

--Fig. 2 shows a base sequence of DNA and a chemical structure of ImPyLDu86 used in the experiment in Fig. 1 (SEQ ID NOS:3-4)--.

DEVELOPMENT OF METHOD FOR SCREENING PHYSIOLOGICALLY ACTIVE PYRROLE IMIDAZOLE DERIVATIVE

Technical Field

The present invention relates to a method for detecting or identifying an action for substances containing DNA or RNA such as cells, using a chemical species having a chemical structure containing non-natural base which can recognize base sequences of natural DNA or RNA, a kit therefor and a plate to be used therefor.

Background Art

As a result of studies conducted by the human genome project, base sequences of the full set of genes, "a draft of the human life", will be elucidated within a few years. It is known that diseases or senescence will be developed if the draft has a defect or is injured. As the results of development of the human genome project, many diseases including cancer can be elucidated in a DNA level, and the total medical sciences consisting mainly of diagnosis and prevention are believed to be changed revolutionarily. Further, although we have a great expectation for developments of a therapeutic method based on understanding in DNA levels of diseases and pharmaceuticals targeting in causal genes of the diseases or their products, studies of such fundamental researches mediating to clinical studies have just started. Antitumor drugs used at present are mainly antibiotics selected by screening works, and originally are not the product produced by microorganisms for the purpose of killing tumor cells. Furthermore few drug based on the molecular biological

knowledge on tumor has been known. If an expression of an intracellular specific gene can be freely controlled extracellularly, an ultimate therapeutic method in a gene level can be achieved.

We, recently, found that an antibiotic duocarmycin could construct a heterodimer with other kinds of molecules such as distamycin to perform cooperatively molecular recognition of DNA and also perform efficiently an alkylation to a base sequence different from the case of duocarmycin alone (Proc. Natl. Acad. Sci. USA 93, 14405, 1996). Based on the results of this study, we succeeded to synthesize a molecule which could selectively alkylate DNA at any position of its base sequence, by binding pyrrole-imidazole polyamide as a recognition site for DNA to the active alkylation site of duocarmycin, and applied a patent (JP-A-10-260710).

However, the compounds, in which the pyrrole-imidazole polyamide as a DNA recognition site is bound only in the active alkylation site of duocarmycin, are not only insufficient in the alkylation activity but also able only to recognize a single-stranded base sequence. Consequently, we examined alkylation mechanisms between these molecules and DNA in detail using a computer modeling such as molecular dynamics of these compounds, and found that double-stranded DNA could be simultaneously alkylated and cleaved by introducing a linker such as vinyl group into the cyclopropane moiety (segment A), an active site of duocarmycin (JP-A-11-83591).

From the fact that these artificial chemical species, which could recognize base sequences of natural DNA and RNA, recognized a specific base sequence of the natural DNA and RNA, and affected an action of the segment A to the specific site of the DNA and RNA, we found that these artificial chemical

species could be applied in place of a partial sequence of the natural DNA and RNA.

Disclosure of the Invention

An aspect of the present invention is to provide a method for screening an action of the segment A (chemical species A) to substances containing DNA or RNA such as cells by using these artificial chemical species.

The present invention relates to a method for detecting or identifying an action of chemical species A to substance containing DNA or RNA comprising using the chemical species, which can recognize a base sequence of the DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing non-natural base which can recognize the base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together chemical structures of A and B.

More particularly, the present invention relates to a method for detecting or identifying an action of chemical species A to a substance containing DNA or RNA comprising providing the compound represented by the general formula (I), which can recognize a base sequence of DNA or RNA in each well of a plate consisting of a plurality of wells, introducing a substance containing DNA or RNA into each well of said plate, reacting completely with the compound represented by the general formula (I) and the substance containing DNA or RNA, and assaying a state of the substance containing DNA or RNA.

More further particularly, in the method described hereinabove, the present invention relates to a method according to the method described hereinabove wherein the compound represented by the general formula (I) in each well is the compound which can recognize a difference in the base sequence of DNA or RNA of the substance containing DNA or RNA and the substance containing DNA or RNA which is introduced into each well is the same substance.

Further, in the method described hereinabove, the present invention relates to a method according to the method described hereinbefore wherein the compound represented by the general formula (I) in each well is the compound which can recognize a specific type of the base sequence of DNA or RNA of the substance containing DNA or RNA and the substance containing DNA or RNA which is introduced into each well is a different substance.

Further, the present invention relates to a kit for detection or identification of an action of the chemical species A to a substance containing DNA or RNA for carrying out the various methods described hereinbefore.

More particularly, the present invention relates to a kit for detecting or identifying an action of the chemical species A to a substance containing DNA or RNA comprising consisting of the chemical species which can recognize a base sequence of the DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing non-natural bases which can recognize a base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together the chemical structures of A and B;

and equipment or reagents for assaying a state of the substance containing DNA or RNA after treatment.

Further, the present invention relates to a plate consisting of a plurality of wells comprising presence of a chemical species which can recognize a base sequence of DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing a non-natural base which can recognize a base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together chemical structures of A and B;

in each well in the plate consisting of a plurality of wells, and relates to a plate comprising a plate for detecting or identifying an action of a chemical species A for a substance containing DNA or RNA.

Brief Description of the Drawings

Fig.1 is a photograph instead of a drawing which shows a result of reaction with ImPyLDu86 of the present invention and DNA

Fig.2 shows a base sequence of DNA and a chemical structure of ImPyLDu86 used in the experiment in Fig. 1.

Fig.3 shows a method for screening antitumor agents specific to tumor cells using the plate of the present invention.

Fig.4 shows survival rates of tumor cells in the concentrations at 100 nM of the compounds 1 - 16 of the present invention.

Fig.5 illustrates a method for testing simultaneously a case using the compounds of the present invention alone and a case using mixture thereof.

Best Mode for Carrying Out the Invention

The chemical structure B containing non-natural bases which can recognize a base sequence of DNA in the general formula (I) hereinbefore of the present invention is preferably the chemical structure derived from pyrrole and/or imidazole which may optionally have substituents. Examples of the substituents of pyrrole and imidazole are not limited so long as they do not hinder a recognition of a base sequence of DNA, and are straight or branched alkyl group having 1-10 carbon atoms, preferably 1-5 carbon atoms including, for example, alkoxy group derived from said alkyl group, hydroxyl group, amino group, N-alkyl-substituted amino group derived from said alkyl group, N-acylamino group derived from organic carboxylic acids, guanidino and substituted guanidine groups. More specifically, N-methylpyrrole, N-methylimidazole, 3-hydroxypyrrole, N-methyl-3-hydroxypyrrole, and the like are included.

These non-natural bases which can recognize a natural base sequence may optionally be located in a main chain or may optionally be pendent from a main chain. When these non-natural bases are located in a main chain, these non-natural bases per se may have functional groups for constructing the main chain, for example, a carboxyl group in an end and an amino group in the other end of the non-natural base, and these bases may construct a polyamide structure. The structure constructing the main chain may not be limited within the above polyamide structure, and can be a structure which constructs polyester structure, polyimine structure, and the like.

When these non-natural bases are pendent from a main chain, these bases may be pendent from a structure of polysaccharide such as natural DNA

or RNA, or may be pendent from a structure of a synthetic polymer.

Preferable example of the chemical structure B containing non-natural bases, which can recognize a base sequence of DNA, is, more specifically, a pyrrole-imidazole polyamide linkage. Numbers (lengths) of pyrrole and imidazole are not limited, and are preferably about 2-30 units, more preferably about 24-16 units, and further more preferably about 4-16 units.

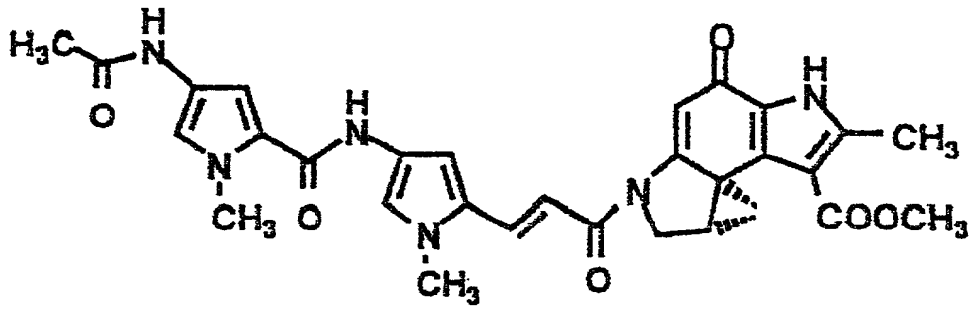
The chemical structure A, which can bind with a base in DNA, can be a variety of chemical species so long as they can interact with DNA or RNA. Example of the preferable structure of the chemical species (segment A) is a structure of chemical substance having an antitumor activity. Example of a chemical substance having an antitumor activity is preferably alkylating agent for acting to DNA. More preferably, it is a chemical structure having a cyclopropane ring, and further more preferably alkylating moiety of duocarmycin.

The linker moiety L which can bind with the chemical structures A and B may preferably be a structure which can separate the segment A and the segment B with a suitable distance as well as does not inactivate the alkylating activity. Preferable example is a chemical structure having a vinyl group.

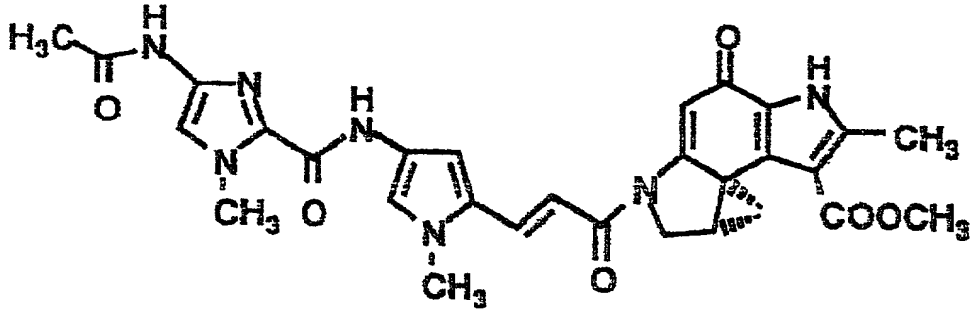
The compound represented by the general formula (I) can be used alone or as a mixture of two or more of these compounds. When two or more compounds represented by the general formula (I) are used as a mixture, there may be various mixing patterns. Generally, a mixture of chemical species having different chemical species B (segment B) from each other may be preferable, but is not limited within the same.

Examples of a preferable compound of the present invention represented

by the general formula (I) are the compound represented by the following formula (hereinafter designated as "PyPyLDu86"):

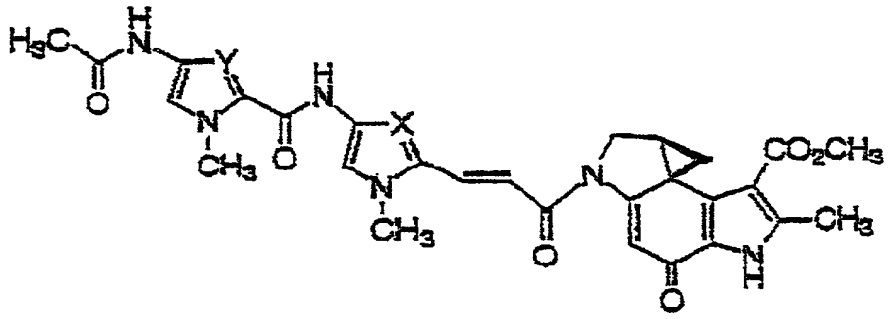


or the compound represented by the following formula (hereinafter designated as "ImPyLDu86"):



The compounds described hereinabove can recognize sequences such as a base sequence TGACG, or a complementary strand thereof corresponding to ImPyLDu86.

Further, a compound represented by the general formula (II) having a structure of {Py or Im}{Py or Im}Ldu86 of the following formula, which is constructed with a basic structure PyPyLDu86:



(II)

wherein X and Y are, each independently, -CH= or -N=,

or a mixture thereof in the ratio of 1 : 1 is included.

For the explanations hereinbelow, these compounds or mixtures thereof are numbered as follows.

A compound wherein X is CH and Y is CH is designated as compound 1;

a compound wherein X is CH and Y is N is designated as compound 2;

a compound wherein X is N and Y is CH is designated as compound 3;

a compound wherein X is N and Y is N is designated as compound 4;

a mixture of the compound 1 and the compound 2 in the ratio of 1 : 1 is designated as compound 5;

a mixture of the compound 1 and the compound 3 in the ratio of 1 : 1 is designated as compound 6;

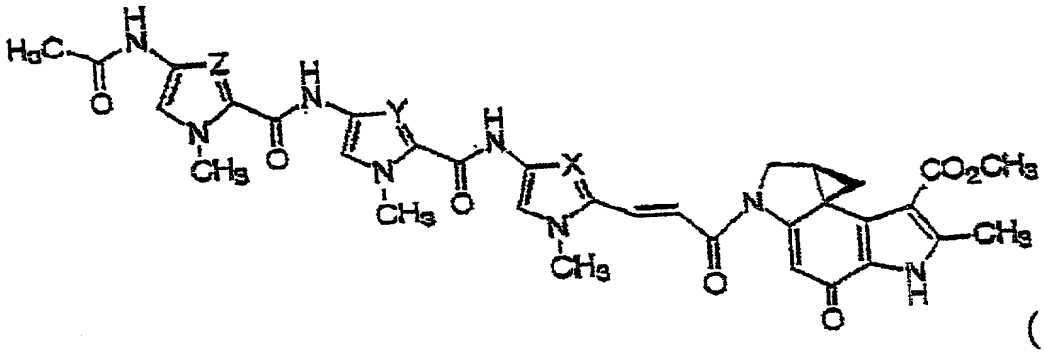
a mixture of the compound 1 and the compound 4 in the ratio of 1 : 1 is designated as compound 7;

a mixture of the compound 2 and the compound 3 in the ratio of 1 : 1 is designated as compound 8;

a mixture of the compound 2 and the compound 4 in the ratio of 1 : 1 is designated as compound 9; and

a mixture of the compound 3 and the compound 4 in the ratio of 1 : 1 is designated as compound 10.

Further, a compound represented by the general formula (III) having a structure of {Py or Im}{Py or Im}{Py or Im}Ldu86 of the following formula, which is constructed with a basic structure PyPyPyLDu86:



(Ⅲ)

wherein X, Y and Z are, each independently, -CH= or -N=,

or a mixture thereof in the ratio of 1 : 1 can be included.

For the explanations hereinbelow, these compounds or mixtures thereof are numbered as follows.

A compound wherein X is CH, Y is N and Z is N is designated as compound 11;

a compound wherein X is CH, Y is N and Z is CH is designated as compound 12;

a compound wherein X is CH, Y is CH and Z is CH is designated as compound 13;

a mixture of the compound 11 and the compound 12 in the ratio of 1 : 1 is designated as compound 14;

a mixture of the compound 11 and the compound 13 in the ratio of 1 : 1 is

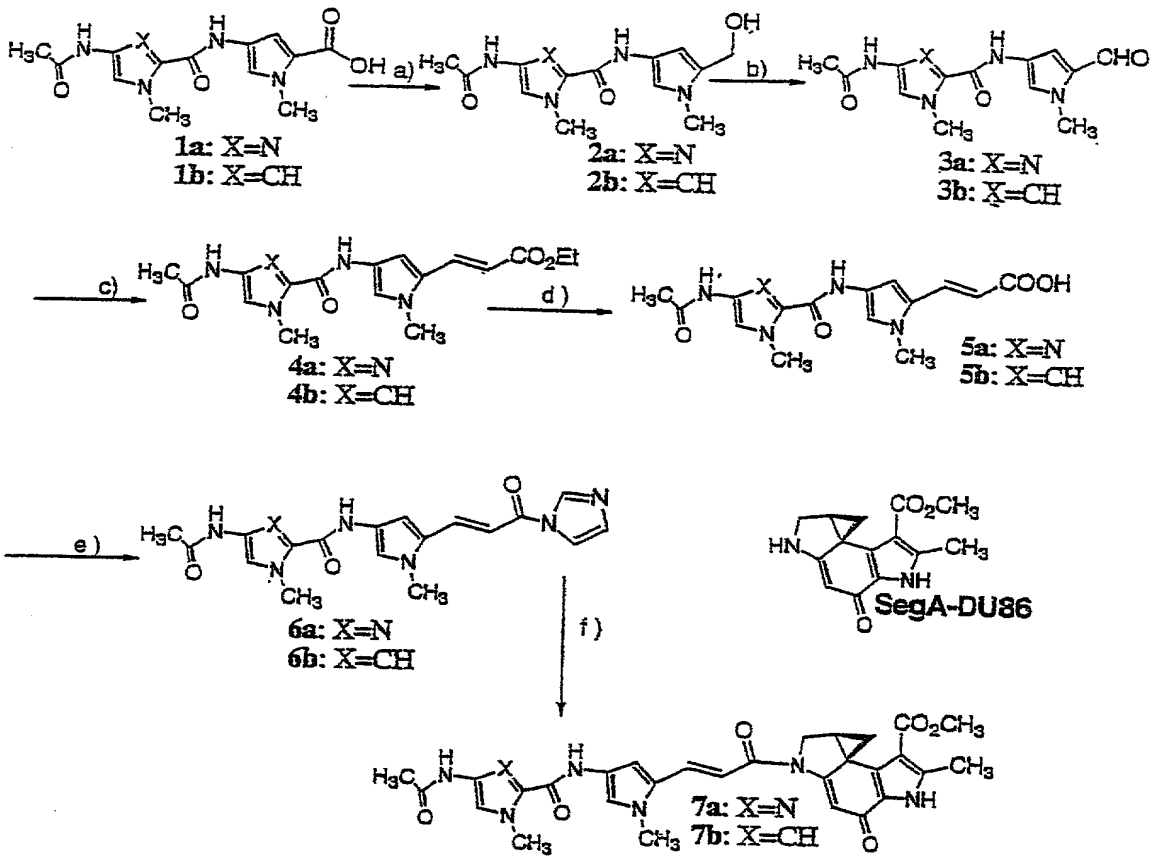
designated as compound 15; and

a mixture of the compound 12 and the compound 13 in the ratio of 1 : 1 is designated as compound 16.

These compounds 1-16 are used in the experiments described hereinbelow.

The compound represented by the general formula (I) can be produced according to the known methods. Namely, the compound can be produced by producing the segment A and the segment B by the conventional methods; binding the linker segment L successively therewith; and then further binding the remaining segments therewith.

For example, examples of the processes for producing ImPyLDu86 (7a) and PyPyLDu86 (7b) described hereinbefore are illustrated in the following chemical reaction scheme. Numbers below each compound in the reaction scheme show the numbers of those compound.



In the reaction scheme; a) indicates treatment with a solution of benzotriazole-1-yl-oxytris (dimethylamino) phosphonium hexafluorophosphate (BOP) in THF, followed by $NaBH_4$ treatment; b) indicates treatment with MnO_2 in THF; c) indicates treatment with triethylphosphonoacetate and NaH in THF; d) indicates treatment with sodium hydroxide in aqueous methanol; e) indicates treatment with 1,1-carbonyl-di-imidazole in DMF; and f) indicates treatment of Du86 with the segment A using sodium hydride in DMF.

Reactivities of thus synthesized PyPyLDu86 and ImPyLDu86 with DNA were determined. Results of alkylation by ImPyLDu86 is shown in Fig.1.

DNA and ImPyLDu86 used in the present experiment are shown in Fig.2.

In Fig.1, the left migration pattern shows a result of the upper strand of the double-stranded DNA, and the right migration pattern shows a result of the lower strand of the double-stranded DNA. Locations of alkylation can be observed as cleavage bands by heating. As a result, the double-stranded DNA was cleaved mainly at the site 1 and the site 2 from a low concentration, and it was understood that the alkylation occurred simultaneously in the double-stranded DNA. No compound to generate such a cleavage has been known until now, and it can be surely regarded as an artificial restriction enzyme. Moreover, it was found that the cleavage occurred in such a high ratio as 70%, showing a very high efficiency in comparison with the previously synthesized compounds (JP-A-10-260710).

As for an example of a substance containing DNA or RNA, it is preferable to use living cells although DNA or RNA per se can be used. When an antitumor agent is used as the segment A, tumor cells can be used.

In a case when methylpyrrole (Py) and methylimidazole (Im) are used as non-natural bases in the segment B in the general formula (I), since it is known that a C-G base pair is recognized by Py-Im; a G-C base pair is recognized by Im-Py; and a A-T or a T-A base pair is recognized by Py-Py, an objective base sequence can be recognized by appropriately combining methylpyrrole (Py) and methylimidazole (Im). Namely, a sequence of natural three bases can be recognized by using three units (trimer) of methylpyrrole (Py) and methylimidazole (Im), and a sequence of natural four bases can be recognized by using four units (tetramer) of methylpyrrole (Py) and methylimidazole (Im).

Furthermore, a compound having a sequence of the segment B can be

used as a mixture of two or more kinds of them.

In the method of the present invention, an actions of the chemical species A to a substance containing DNA or RNA can be detected or identified by assaying a state of the substance containing DNA or RNA, after reacting completely the compound represented by the general formula (I) with a substance containing DNA or RNA.

As a mean for reacting completely the compound represented by the general formula (I) with a substance containing DNA or RNA, the reaction can be performed by incubating both in a suitable buffer. As a mean for assaying a state of a substance containing DNA or RNA after the incubation, various labeling or coloring methods can be used. These means can be suitably selected depending on a state of a substance containing DNA or RNA.

When living cells are used as a substance containing DNA or RNA, and their state is detected by their survivals, a method for coloring cells is simple and preferable. Quantification of numbers of living cells can be achieved by using commercially available cell counting kit or by combining the kit with a light absorbance in the coloring.

Embodiments of use of the present invention will be explained concretely in the following.

Fig.3 illustrates a method of the present invention. The left figure in Fig.3 shown like a section paper is a plate consisting of a plurality of wells, and each square indicates each well. In this example, a plate with 96 wells is illustrated. In each well, the chemical species represented by the general formula (I) hereinbefore of the present invention are present. Firstly, a case will be explained wherein the chemical species represented by the general formula

(I) of the present invention in each well recognizes different base sequence from each other.

In the case of a tetramer, for example, non-natural bases consisting of methylpyrrole (Py) and methylimidazole (Im) are used in the moiety of the segment B in the chemical species represented by the general formula (I), and a set of bases, which can recognize different three bases, can be prepared by using the structures of all permutations and combinations consisting of Py and Im such as Py-Py-Py-Py, Py-Py-Py-Im, Py-Py-Im-Py and Im-Im-Im-Py (in this case, 16 varieties of combinations can be obtained). An alkylating agent is bound to the segment A of the general formula (I), then the resulting compound is linked with the linker L containing vinyl group.

Then, sixteen types of chemical species having different structures in the segment B are added into each well of the plate shown in the left figure in Fig.3. Subsequently, tumor cells are added to each well, and incubated for several hours to several days. As a result, the chemical species represented by the general formula (I) recognize a specific region of the base sequences of the tumor cells and reacts with the DNA of tumor cells and alkylating them to kill the tumor cells. In the right figure in Fig.3, contents of each well are stained after completing the above incubation. Living cells are stained by coloring agents as shown black color in Fig.3, whereas dead cells can not be stained as shown blank (white) in Fig.3. The example shown in Fig.3 is the case in which octamer is used as the segment B, and it is shown that the cancer cells are killed in three types of non-natural base sequences. Since the non-natural base sequence present in each well is known in advance, it can be known by this test in what cases of sequences the tumor cells are killed.

In the case shown in Fig.3, an octamer is used. Consequently, the chemical species represented by the general formula (I) having 2^8, i.e. 256 types of the segment B moieties are present in each well. In this case, it is observed that three types of sequences among them can specifically kill the tumor cells. In this case, the three sequences are found to be as follows:

PyImPyPyPyImPyPy,

PyImPyPyPyImImPy, and

ImImPyPyPyImPyPy.

Since tumor cells are different depending on their types and organisms, according to the method described hereinbefore in the present invention, antitumor agents having specific action to the tumor cells as a specimen assayed can be retrieved within a short time in a simple manner. Further, tumor cells, even in the same tissue, may be mutated depending on their stage. Even in such case, an antitumor agent specific to the mutated tumor cells can conveniently be retrieved by the method of the present invention. In addition, according to the method of the present invention described hereinbefore, an action of the antitumor agent to peripheral normal cells of the tumor tissues can also be studied by the same method.

Consequently, the method of the present invention provides a convenient screening method for an antitumor agent having a specific action to the objective tumor cells without affecting normal cells within a short time.

Then, the compounds 1-16 mentioned hereinbefore were assessed for their activities.

Cytotoxicity tests were performed using compounds consisting of 2 or 3 pyrrole (Py) and imidazole (Im) amide moieties in total. Results of effects

indicated by survival rates of cells using the compounds 1-16 described hereinbefore are shown in Fig.4. Simultaneous screening tests using human tumor cells LCL-wt, HLC-2 and human leukemia cell Jurkat showed that only the compound 14 showed high cytotoxicity against LCL-wt and no useful effect was shown against Jurkat and HLC-2.

Fig.4 shows the test results on cytotoxicities of the compounds 1-16 against LCL-wt and Jurkat at the concentration of 100 nM.

As shown above, it became obvious that mixtures of 2 or more compounds represented by the general formula (I) of the present invention showed specific activities. Cases of the test methods using these mixtures are shown in Fig.5. In Fig.5, a test using $8 \times 8 = 64$ wells are shown.

Cases with 3 recognition sites in the segment B are illustrated. When pyrrole (Py) and imidazole (Im) are used as the recognition components, combinations of 2^3 types, i.e. 8 types, can be included. These 8 types, 25 μl each, were added to each well in the lengthwise and the breadthwise. For example, 8 types of compounds were added to each line and row of wells, respectively, in such manner as adding Py-Py-Py, 25μl each, on the first line and the first row of the plate, then adding Py-Im-Py, 25μl each, on the second line and the second row of the plate, further adding Py-Py-Im, 25μl each, on the third line and the third row of the plate. As a result, only one type of the compound was added in the wells on the diagonal line of the plate, and the mixtures consisting of 2 types of compounds in a ratio of 1:1 were added in the wells other than those on the diagonal line. Results of incubation with tumor cells performed by the same method as the method shown in Fig.3 and treatment with coloring agents are illustrated in Fig.5.

In the case shown in Fig.5, death of the tumor cells are observed in the well on the third line and the third row, wells on the second line and the sixth row and on the sixth line and the second row, and wells on the fourth line and the eighth row and on the eighth line and the fourth row. Since the well on the third line and the third row is on the diagonal line, Py-Py-Im alone can be found to kill the tumor cells. Since the wells on the second line and the sixth row and on the sixth line and the second row and the wells on the fourth line and the eighth row and on the eighth line and the fourth row are located in the symmetrical positions about the diagonal line, the former is a 1 : 1 mixture of Py-Im-Py and Im-Py-Im and the latter is a 1 : 1 mixture of Py-Im-Py and Im-Im-Im. In this case, it is shown that the compound or the mixtures having these segments B are specifically effective against these tumor cells.

Further, this experimental case demonstrates an important point that although an efficacy of the compound of the general formula (I) against tumor cells is not shown when the compound is used alone, there is a case showing an efficacy only when used in a mixture with other compound.

According to the test method of the present invention, results of the case using the compound represented by the general formula (I) alone can be obtained, and simultaneously results of the case using these compounds as a mixture can also be obtained.

The above case is the method for retrieving an antitumor agent specific to tumor cells. Further, a chemical species having the segment B moiety represented by the general formula (I) of the present invention corresponding to said base sequence is prepared using tumor cells, of which an efficacy is known at a specific location in the base sequence, as the substance containing DNA or

RNA, a plurality of types of chemical species represented by the general formula (I) of the present invention, which are bound with various candidate compounds of an antitumor agent and different each other in their segment A moieties, are provided in each well, then these chemical species are incubated with tumor cells described hereinbefore, thus actions of the candidate compounds in the segment A moieties can be retrieved.

An aspect of the present invention is to provide a screening method for an antitumor action to tumor cells by binding the candidate compound of an antitumor agent to the segment A moiety of the general formula (I) of the present invention.

Further, the present invention provides a kit for detecting or identifying an action of the chemical species A for the substance containing DNA or RNA for performing the various methods of the present invention described hereinbefore.

More particularly, the present invention provides a kit for detecting or identifying an action of the chemical species A to the substance containing DNA or RNA comprising consisting of the chemical species, which can recognize a base sequence of DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing non-natural bases which can recognize a base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together chemical structures of A and B;

and equipment or reagents for assaying a state of the substance containing DNA or RNA after treatment. As described hereinbefore, the compound of the

general formula (I) of the present invention may be used alone or may be prepared to be used as a mixture of 2 or more types of the compounds.

Further, the present invention provides a plate consisting of a plurality of wells comprising providing the chemical species, which can recognize a base sequence of DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing non-natural bases which can recognize a base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together chemical structures of A and B;

in each well in the plate consisting of a plurality of wells. More particularly, the plate of the present invention is a plate for detecting or identifying an action of the chemical species A to the substance containing DNA or RNA.

The compound represented by the general formula (I) hereinbefore may be of one type or two or more types, being immobilized in each well of the plate. These compounds may also be in a state of solution or gel.

The present invention will be explained in detail by concrete examples, but is not limited within these concrete examples.

Examples

Example 1 (Assessment of antitumor effect by cytotoxicity test)

Human tumor cells LCL-wt and HLC-2, human leukemia cell, Jurkat, and human cervix cancer cell HeLa cell were used as tumor cells. For LCL-wt

and Jurkat, PRMI 1640 (Gibco BRL) + 10% fetal bovine serum (JRH BIOCIENCES) + 100 μU/ml penicillin G - 100 μU/ml streptomycin sulfate (Gibco BRL) were used as the medium for incubation. For HLC-2, MEM + 10% fetal bovine serum (JRH BIOCIENCES) + 100 μU/ml penicillin G - 100 μU/ml streptomycin sulfate (Gibco BRL) were used as the medium for incubation. For HeLa cell, RBMI + 10% fetal bovine serum (JRH BIOCIENCES) + 100 μU/ml penicillin G - 100 μU/ml streptomycin sulfate (Gibco BRL) were used as the medium for incubation. Respective cells were incubated and cells with the logarithmic growth phase were suspended and used for screening.

The screening was carried out as follows. Cell suspension adjusted to the initial cell counts at approximately 2×10^5 cells/ml was added separately into 96 wells of multi-plate at 50 μl/well. The test solution of the test compound (100 μM, medium + 0.1% DMSO)was added thereto and incubated at 37℃, under 5% CO_2 concentration, for 2 days in the incubator, then cell counts were counted.

Cell counts were calculated using a micro plate reader (MPR-A4i, TOSOH) and a hemocytometer. In an assay using the micro plate reader, the cell counting kit-8 (DOJINDO) was used, and light absorbance was measured at 450 nm (reference wave length 600 nm). Viable cell counts and inviable cell counts were performed by the dye exclusion test using trypan blue under a microscope. Based on the results of measuring using the micro plate reader and the hemocytometer, survival rate was calculated by the following formula:

Survival rate = $100\ n_p/n_a$

wherein n_p is the viable counts with addition of sample and n_a is the control viable counts.

Example 2 (Cytotoxicity test)

Simultaneous screening tests were performed using human tumor cells LCL-wt, HLC-2 and human leukemia cell Jurkat with the plate of the present invention using the compounds 1-16 described hereinbefore.

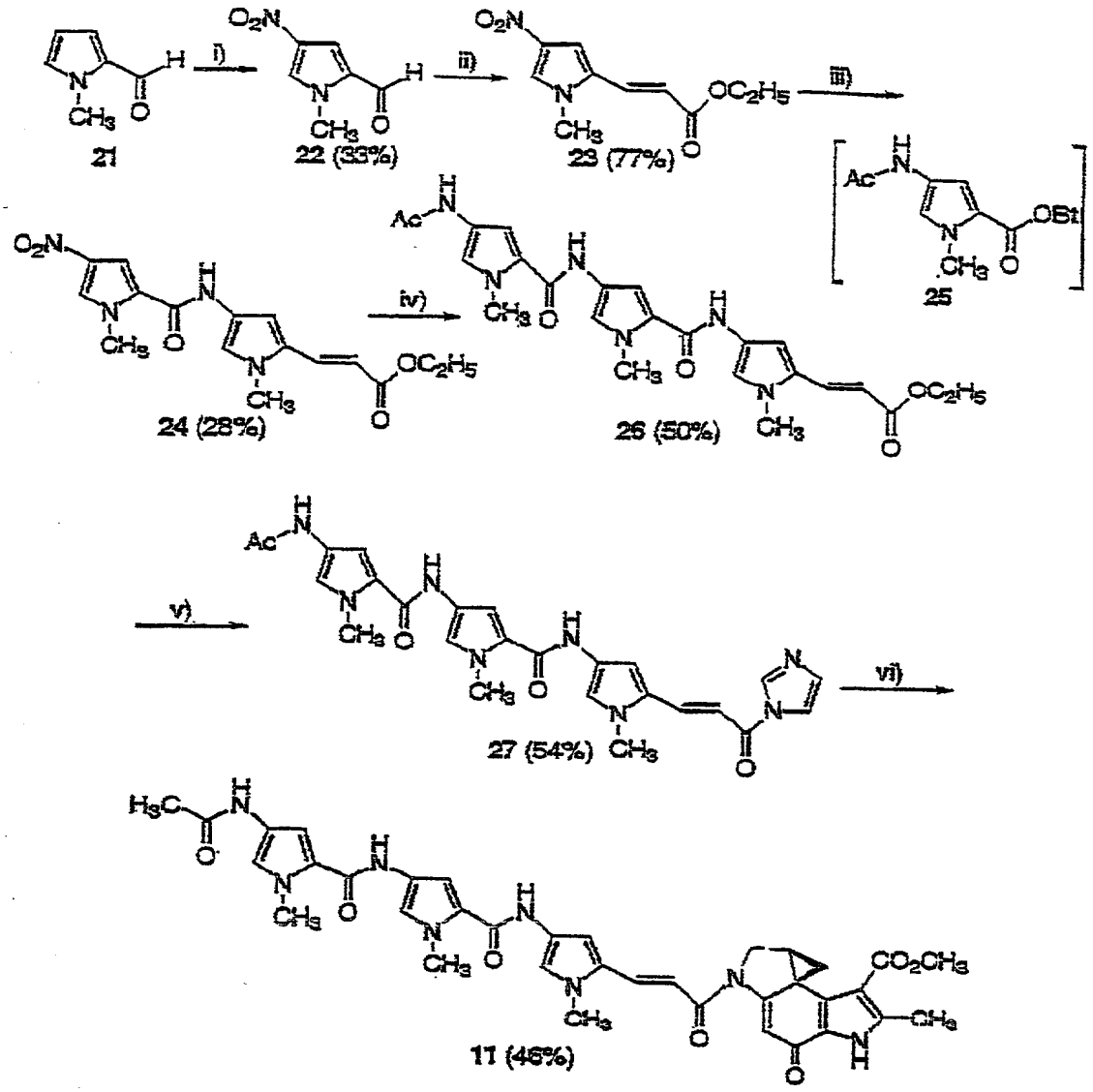
Results were treated in the same way as in example 1 and survival rate of each cell was calculated.

In Fig.4, results of the test compounds at the concentration of 100 nM are shown.

The results indicate that the compound 14 shows a high cytotoxic action only against LCL-wt.

Example 3 (Synthesis of compounds)

The method of synthesis of the compound 13 is illustrated as follows.



The commercially available materials were used for reagents used in the reactions and the purifications and solvents. For proton nuclear magnetic resonance spectra (NMR), Nihon Denshi JNM-A500 was used. Tetramethylsilane (TMS) was used as an internal standard substance and chemical shifts were shown by δ-value (ppm). Abbreviations for signals are shown as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad) and br s (broad singlet). Abbreviations of reagents and solvents are

as follows: dimethylformamide (DMF), dicyclohexylcarbodiimide (DCC), carbonyldiimidazole (CDI), 4-(dimethyl) aminopyridine (DMAP), N-hydroxybenzotriazole (HOBt), 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and tetrahydrofuran (THF). The reactions were performed, if not specified, under an argon atmosphere or a nitrogen atmosphere.

(1) 1-methyl-4-nitro-pyrrole-2-aldehyde (22)

Acetic anhydride (25 ml) solution of fuming nitric acid (1.5 ml, 37.5 mmol) was cooled at -30 ℃. Acetic anhydride (10 ml) solution of 1-methylpyrrole-2-carboxyaldehyde (21) (3.27 g, 30.0 mmol) was dropwisely added under the same temperature, and the mixture was stirred at the same temperature for 5 hours. The precipitated solid was filtered to obtain nitro compound (22) (860 mg, 19%). The solvent of the filtrate was removed in vacuo, and the obtained residue was charged on a silica gel column chromatography to obtain an additional (22) (650 mg, 14%) from hexane-ethyl acetate (4 : 1, v/v) eluate.

^{1}H NMR ($CDCl_3$) δ : 4.00(3H,s), 7.40(1H,d,J=2.0Hz), 7.65(1H,d,D=2.0Hz), 9.61((1H,s);

IR (KBr) ν: 1678, 1535, 1508, 1423, 1406, 1311, 1100, 864, 814, 770, 754cm^{-1}

(2) Py-L-CO_2Et (23)

Sodium hydride (83 mg, 2.1 mmol) was added to THF (15 ml) solution of triethyl phosphonoacetate (0.39 ml, 2.0 mmol) under ice cooling and stirred for

10 minutes. THF (5 ml) solution of nitro compound (22) was dropwisely added at the same temperature, and stirred at the same temperature for further 45 minutes. Water was added to the reaction mixture and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride solution and dried by adding anhydrous magnesium sulfate. The solvent was distilled off in vacuo, and the obtained residue was charged on a silica gel column chromatography to obtain ester (23) (225 mg, 77%) from hexane - ethyl acetate (1 : 4, v/v) eluate.

^{1}H NMR ($CDCl_3$) δ : 1.28(3H,t,J=7.5Hz), 3.75(3H,s), 4.24(2H,q,J=7.5Hz), 6.28(1H,d,J=16.0Hz), 7.09(1H,d,J=2.0Hz), 7.47(1H,d,J=16.0Hz), 7.54(1H,d,J=2.0Hz);

^{13}C NMR ($CDCl_3$) δ : 14.3, 35.4, 60.8, 106.1, 118.4, 125.3, 129.8, 130.1, 136.7, 166.5;

IR (KBr) ν : 1709, 1632, 1510, 1427, 1412, 1373, 1315, 1282, 1176cm^{-1}

(3) Py- Py-L-CO_2Et (24)

To methanol solution (45 ml) of the ester (23) (1.12 g, 5.0 mmol), 10% palladium carbon (250 mg) was added at room temperature. 1 N-sodium borohydride (8 ml) was added to the mixture at the same temperature and stirred for further 10 minutes. After addition of acetone (2 ml), the suspension was passed through Celite and the precipitate was removed. The solvent of the filtrate was distilled off in vacuo and ethyl acetate was added to the obtained residue. The solution was washed with aqueous saturated sodium chloride solution and dried by adding anhydrous magnesium sulfate. The solvent was distilled off in vacuo. The obtained residue was dissolved in methylene

chloride (45 ml) and was used for the subsequent reaction. 1-methyl-4-nitro-2-trichloroacetylpyrrole (2.35 g, 7.0 mmol) and N,N-diisopropylethylamine (1.31 ml, 7.5 mmol) were gradually added to the solution at room temperature and stirred at the same temperature for 3 hours. Water was added to the reaction mixture and extracted with ethyl acetate. The organic layer was washed with aqueous saturated sodium chloride solution and dried by adding anhydrous magnesium sulfate. The solvent was distilled off in vacuo. The obtained residue was charged on a silica gel column chromatography to obtain bispyrrole (24) (483 mg, 28%) from the ethyl acetate eluate.

^{1}H NMR ($CDCl_3$+DMSO-d_6) δ : 1.23(3H,t,J=7.0Hz), 3.59(3H,s), 3.94(3H,s), 4.14(2H,q,J=7.0Hz), 6.01(1H,d,J=15.5Hz), 6.57(1H,d,J=2.0Hz), 7.27(1H,br s), 7.45(1H,d,J=15.5Hz), 7.46(1H,d,J=1.5Hz), 7.50(1H,d,J=2.0Hz), 9.59(1H,br s)

(4) Py- Py- Py-L-CO_2Et (26)

To the suspension of bispyrrole (24) (173 mg, 0.50 mmol) in methanol - ethyl acetate (10 ml - 10 ml), 10% palladium carbon (50 mg) was added at room temperature. 1 N sodium borohydride (1.5 ml) was added to the mixture at the same temperature and stirred for further 2 minutes. The suspension was passed through a silica gel column chromatography to remove the precipitate. The solvent was distilled off in vacuo. The obtained residue was dissolved in DMF (10 ml) and was used for the subsequent reaction. 4-acetamino-1-methylpyrrole-2- carboxylate HOBt ester (25) [Z. -F. Tao, et al., J. Am. Chem. Soc., 121, 4961 - 4967 (1999)] (209 mg, 0.70 mmol) and DMAP (85 mg, 0.70 mmol) were gradually added to the solution and the mixture was stirred at the

same temperature for 3 hours. The solvent was distilled off in vacuo and the obtained residue was subjected to a silica gel column chromatography. Tris-pyrrole (26) (120 mg, 50%) was obtained from methanol - ethyl acetate (1 : 9, v/v) eluate.

^{1}H NMR ($CDCl_3$) δ : 1.29(3H,t,J=7.0Hz), 2.06(3H,s), 3.59(3H,s), 3.81(3H,s), 3.84(3H,s), 4.20(2H,q,J=7.0Hz), 5.99(1H,d,J=15.5Hz), 6.51(1H,s), 6.58(1H,s), 6.61(1H,s), 6.94(1H,d,J=2.0Hz), 7.13(1H,s), 7.32(1H,d,J=2.0Hz), 7.47(1H,d,J=15.5Hz), 7.78(1H,s), 7.98(1H,s), 8.36(1H,s)

(5) Py- Py- Py-L-CO_2Im (27)

Aqueous 1 N sodium hydroxide solution (1.5 ml) was added to methanol - THF (10 ml - 10 ml) solution of tris-pyrrole (26) (24 mg, 0.050 mmol) at room temperature, and stirred at room temperature for 5 hours. The solvent was distilled off in vacuo. Aqueous 10% acetic acid solution was added to the obtained residue and the precipitate was filtered to obtain the hydrolysate (13.5 mg). The hydrolysate was used in the next reaction step without further purification. CDI (24.3 mg, 0.15 mmol) was added to DMF (1.5 ml) solution of the hydrolysate (12.8 mg) at room temperature and stirred at the same temperature overnight. Water was added and the precipitate was filtered to obtain imidazole ester (27) (13.5 mg, 54%).

^{1}H NMR (DMSO-d_6) δ : 1.96(3H,s), 3.77(3H,s), 3.82((3H,s), 3.85(3H,s), 6.85(1H,s), 7.08(1H,s), 7.09(1H,s), 7.12(1H,d,J=15.0Hz), 7.14(1H,s), 7.22(1H,s), 7.24(1H,s), 7.47(1H,s), 7.87(1H,d,J=15.0Hz), 7.90(1H,s), 8.66(1H,s), 9.80(1H,s), 9.89(1H,s), 10.03(1H,s)

(6) Py- Py- Py-L-Du86 (11)

60% sodium hydride (2.0 mg, 0.050 mmol) was added to DMF (2 ml) solution of Du86 A segment (28)[S. Nakamura, et al., J. Med. Chem., 40, 972-979 (1999)] (6.2 mg, 0.024 mmol) under ice cooling and stirred at the same temperature for 10 minutes. Then DMF (1 ml) solution of imidazole ester (27) (12.9 mg, 0.026 mmol) was added at the same temperature and stirred at the same temperature for further 5 hours. After adding sodium phosphate buffer (pH 6.86), water was added and extracted with methylene chloride. The solvent was distilled off in vacuo and the obtained residue was subjected to a silica gel column chromatography. Py- Py- Py-L-Du86 (11) (7.7 mg, 46%) was obtained from methanol - chloroform (1 : 9, v/v) eluate.

^{1}H NMR (DMSO-d_6) δ : 1.29-1.31(1H,m), 1.97(3H,s), 2.07-2.11(1H,m), 2.47(3H,s), 3.72(3H,s), 3.78(3H,s), 3.82(3H,s), 3.83(3H,s), 4.17-4.22(1H,m), 4.27-4.32(1H,m), 6.57(1H,d,J=15.0Hz), 6.83-6.85(br s), 6.86(1H,s), 6.90(1H,s), 7.06(1H,s), 7.15(1H,s), 7.24(1H,s), 7.39(1H,s), 7.57(1H,d,J=15.0Hz), 9.80(1H,s), 9.89(1H,s), 9.94(1H,s), 12.36(1H,s)

Industrial Applicability

The present invention provides a method for screening a substance specifically acting to specific cells by a simple method within a short time with a high sensitivity as well as by means of an inexpensive mean, a kit and a plate therefor. According to the method of the present invention, drugs acting specifically to cells of patients, for example tumor cells, can be known within a short time. Consequently, tailor-made drugs for treatment depending on tumor cells of an individual patient can be created, and curative drugs with less

side effect and a high efficacy for the patient can be provided

Further, according to the method of the present invention, substances acting to DNA or RNA can be screened conveniently highly sensitively and inexpensively. In addition, a site of action in DNA and RNA of the substance, in which an action to DNA or RNA has been known, can easily be known by the method of the present invention.

CLAIMS

1. A method for detecting or identifying an action of a chemical species A to a substance containing DNA or RNA comprising using the chemical species, which can recognize a base sequence of DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing non-natural bases which can recognize a base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together chemical structures of A and B.

2. The method according to claim 1, for detecting or identifying an action of a chemical species A to a substance containing DNA or RNA comprising providing the compound represented by the general formula (I), which can recognize a base sequence of DNA or RNA in each well of a plate consisting of a plurality of wells, introducing the substance containing DNA or RNA into each well of said plate, reacting completely the compound represented by the general formula (I) with the substance containing DNA or RNA, and assaying a state of the substance containing DNA or RNA.

3. The method according to claim 2, wherein the compound represented by the general formula (I) present in each well is the compound which can recognize a difference of the base sequence of DNA or RNA of the substance containing DNA or RNA and the substance containing DNA or RNA which is introduced into each well is the same substance.

4. The method according to claim 2, wherein the compound represented by

the general formula (I) present in each well is the compound which can recognize specific one type of base sequence of DNA or RNA of the substance containing DNA or RNA, and the substance containing DNA or RNA which is introduced into each well is the different substance.

5. The method according to any of claims 1-4, wherein the compound represented by the general formula (I) is immobilized in the well.

6. The method according to any of claims 1-5, wherein the chemical structure containing non-natural bases, which can recognize a base sequence of DNA or RNA, is the chemical structure which can recognize at least 2 successive bases in natural DNA or RNA of the substance containing DNA or RNA.

7. The method according to any of claims 1-6, wherein the chemical structure containing non-natural bases, which can recognize a base sequence of DNA, is the chemical structure derived from pyrrole and/or imidazole optionally having substituents.

8. The method according to claim 7, wherein the chemical structure derived from pyrrole and/or imidazole optionally having substituents is located in a main chain or is pendent from a main chain.

9. The method according to any of claims 1-8, wherein A having the chemical structure interacting with DNA is a chemical structure of an antitumor agent.

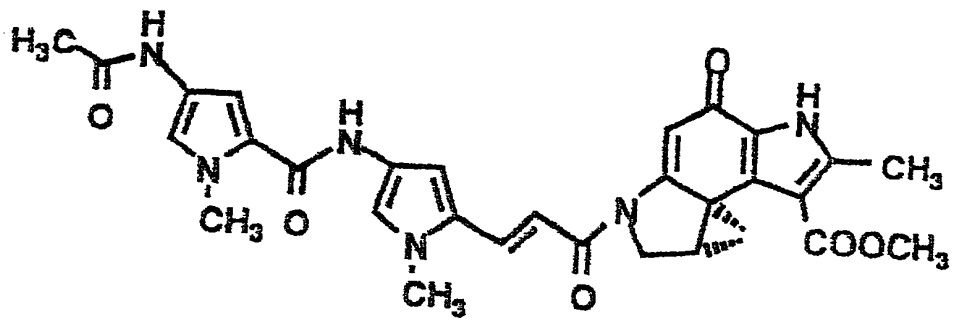
10. The method according to claim 9, wherein the antitumor agent is an alkylating agent.

11. The method according to claim 10, wherein the alkylating agent has a chemical structure having a cyclopropane ring.

12. The method according to any of claims 1-11, wherein the linker, which

can link together the chemical structures of A and B, has a chemical structure containing a vinyl group.

13. The method according to any of claims 7-12, wherein the compound represented by the general formula (I) is the compound represented by the formula:



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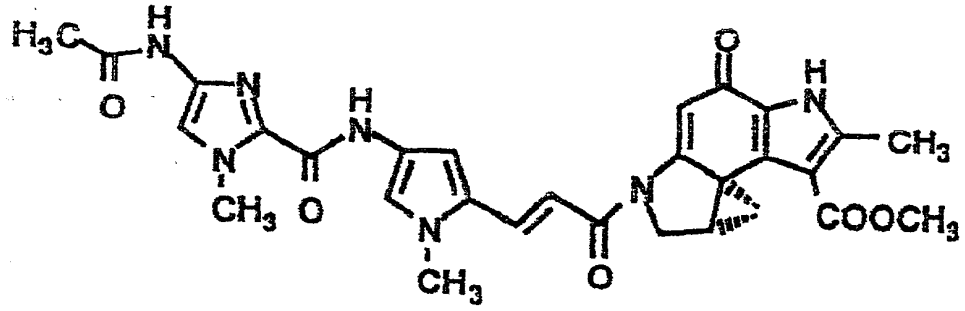


14. The method according to any of claims 1-13, wherein the substance containing DNA or RNA is a cell.

15. The method according to claim 14, wherein the cell is a tumor cell.

16. The method according to any of claims 2-15, wherein a mean for assaying a state of the substance containing DNA or RNA is a method for detecting survival or death of the substance.

17. The method according to claim 16, wherein the method for detecting

survival or death of the substance is coloring of the substance.

18. A kit for detecting or identifying an action of a chemical species A to a substance containing DNA or RNA to perform the method according to any of claims 1-17.

19. The kit according to claim 18 comprising a chemical species, which can recognize a base sequence of DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing non-natural bases which can recognize a base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together chemical structures of A and B;

and equipment or reagents for assaying a state of the substance containing DNA or RNA after treatment.

20. A plate consisting of a plurality of wells comprising presence of a chemical species, which can recognize a base sequence of DNA, represented by the general formula (I):

B-L-A (I)

wherein B is a chemical structure containing non-natural bases which can recognize a base sequence of DNA, A is a chemical structure having an interaction with DNA, and L is a linker which can bind together chemical structures of A and B;

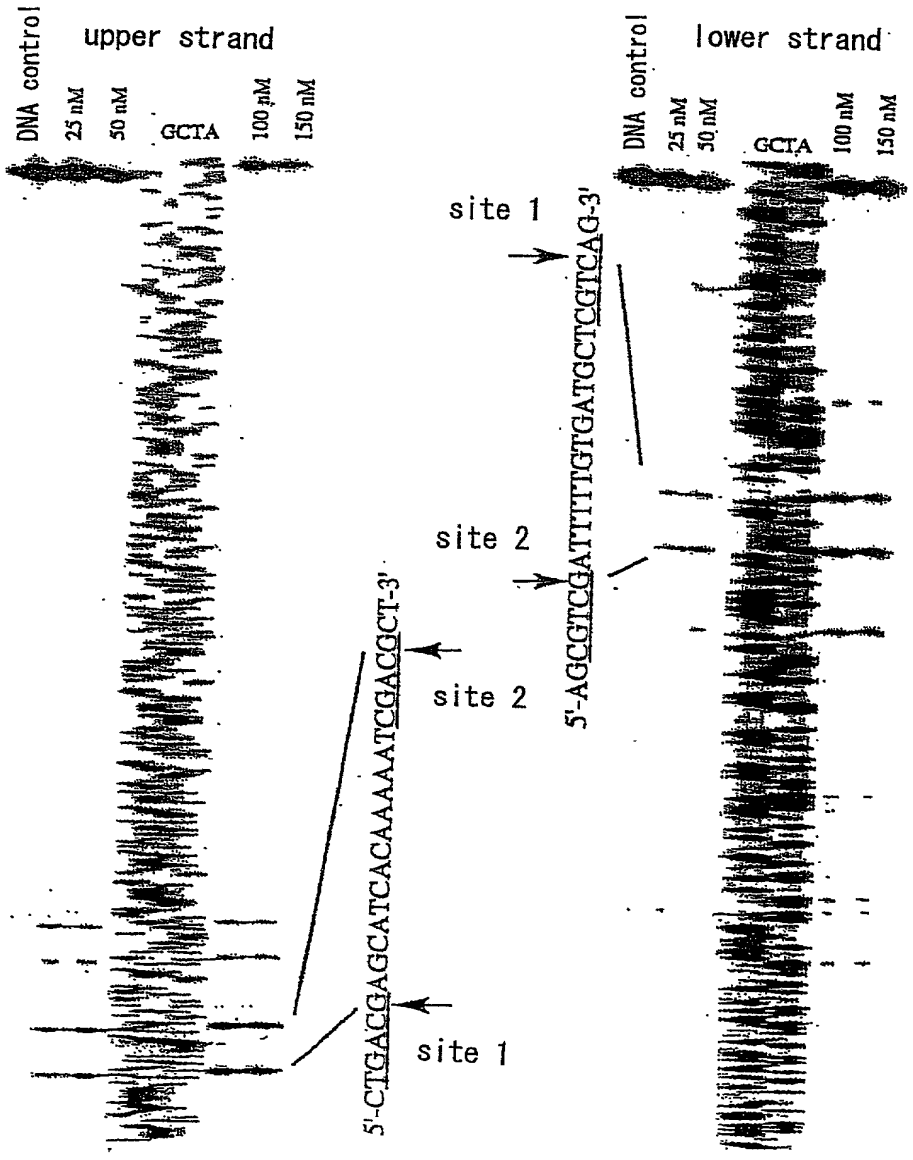
in each well in the plate consisting of a plurality of wells.

21. The plate according to claim 20, comprising a plate for detecting or identifying an action of a chemical species A to a substance containing DNA or RNA.

ABSTRACT

A method for screening the effect of a segment A (chemical species A) on a substance (for example, a cell) containing DNA or RNA by using artificial chemical species. Namely, a method of detecting or identifying the function of a chemical species A on a substance containing DNA or RNA by using one or more chemical species represented by the following general formula (I) which are capable of recognizing a DNA base sequence; a kit therefor; and a plate to be used therein: B-L-A (I) wherein B represents a chemical structure containing an non-natural base capable of recognizing a DNA base sequence; A represents a chemical structure having an interaction with DNA; and L represents a linker whereby the chemical structures A and B can be linked together.

Fig. 1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50 nM
GCTA
100 nM
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site 1
site 2
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site 1
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GCTA
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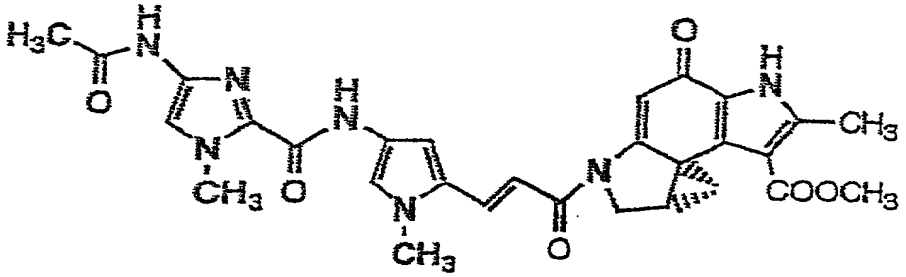
Fig. 2



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site 1 site 2

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Fig. 3

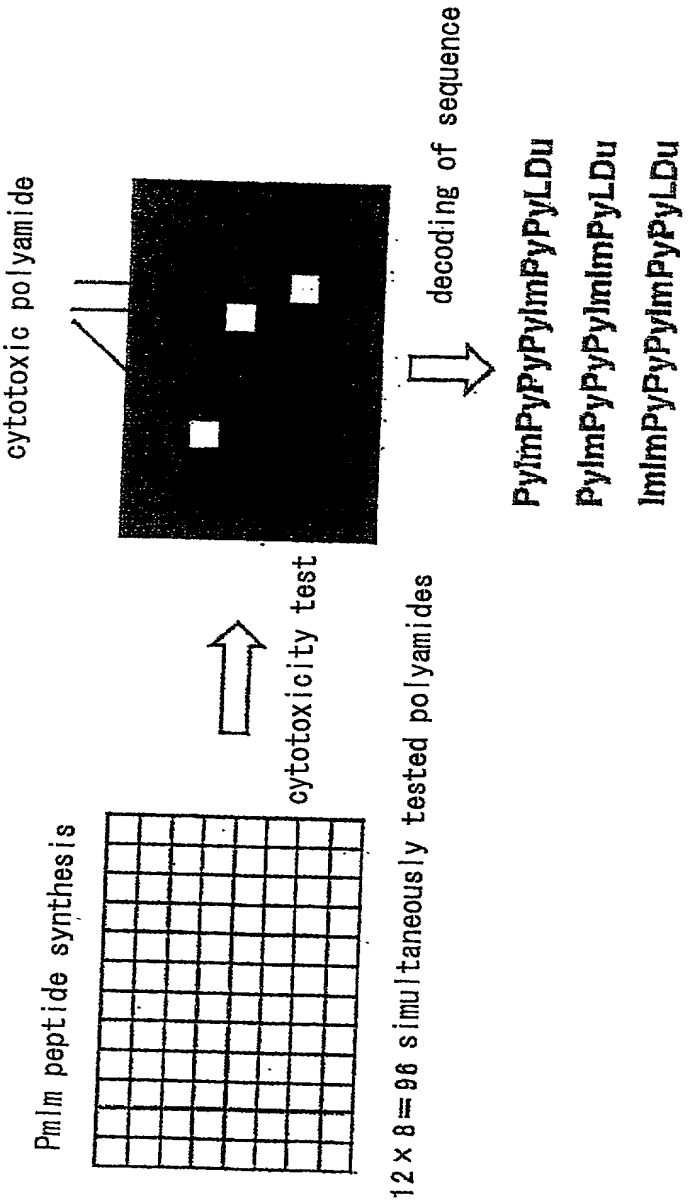
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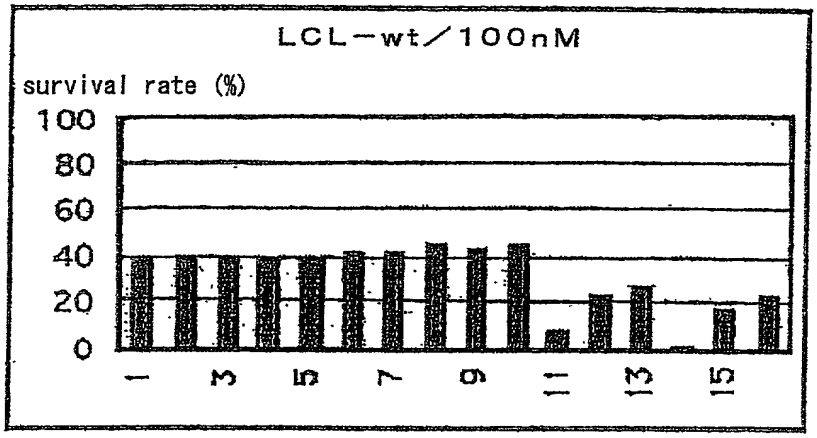
Fig. 4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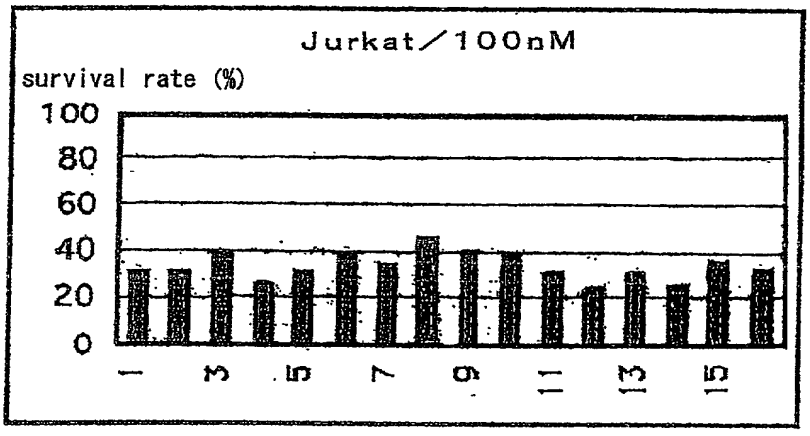
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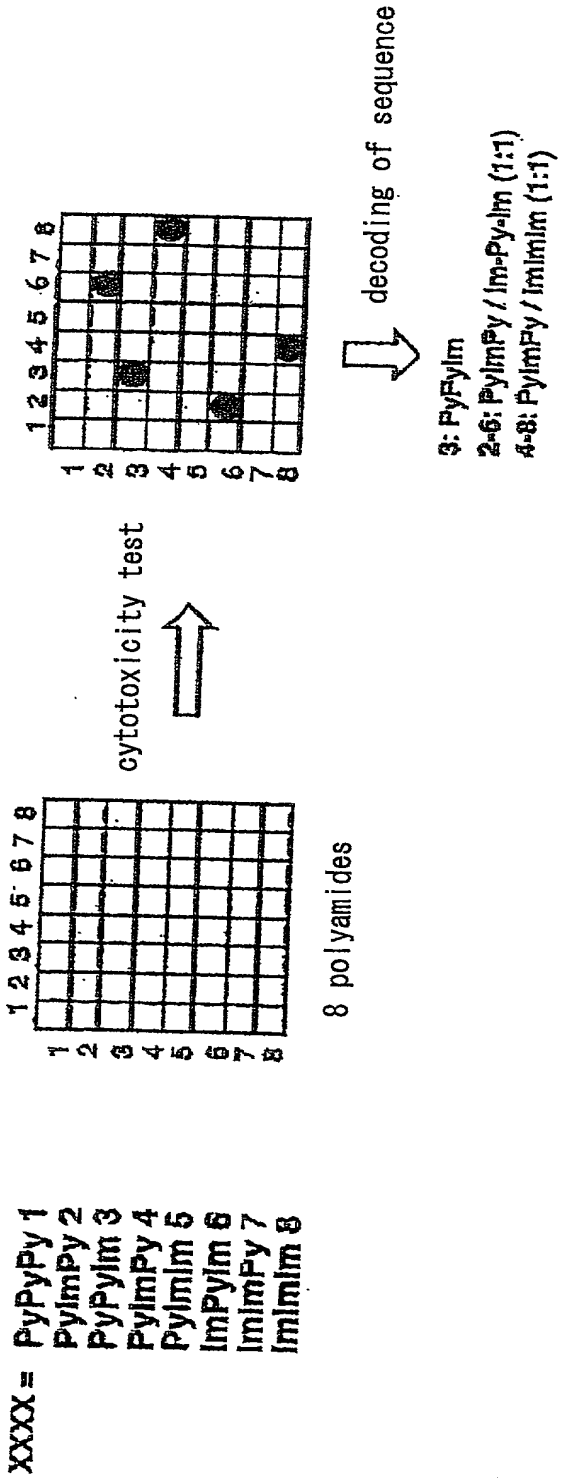
Fig. 5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4-8: PyImPy / ImImIm (1:1)

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Docket No. 56212

PP309460

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DEVELOPMENT OF METHOD FOR SCREENING PHYSIOLOGICALLY ACTIVE PYRROLE IMIDAZOLE DERIVATIVE

which is described and claimed in:

☐ the specification attached hereto.

☒ the specification in the U.S. patent application which corresponds to and claims priority from International Application No. PCT/JP00/07992, filed on November 13, 2001.

☐ the specification in PCT international application Number, filed on _____; and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. §119:			
Application No.	**Filing Date**	**Country**	**Priority Claimed Under 35 U.S.C. §119?**
11-326007/1999	November 16, 1999	Japan	☒YES ☐NO
			☐YES ☐NO
			☐YES ☐NO

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S-Benefit Under 35 U.S.C. §120				
U.S. Applications		Status (Check One)		
Application Serial No.	U.S. Filing Date	Patented	Pending	Abandoned
PCT Applications Designating the U.S.				
Application No.	Filing Date	U.S. Serial No. Assigned		
PCT/JP00/07992	November 13, 2000	Not yet assigned		X

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S) (35 U.S.C. §119(e))

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	Provisional Application Number	Filing Date

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

16—

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Christine C. O'Day	(Reg. No. 38,256)	Steven M. Jensen	(Reg. No. 42,693)	Stuart L. Gitler	(Reg. No. 31,256)
Peter J. Manus	(Reg. No. 26,766)	Lisa Hazzard Swiesz	(Reg. No. 44,358)	Kathryn A. Piffat	(Reg. No. 34,901)
John B. Alexander	(Reg. No. P-48,399)				

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1-00

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	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE

205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE

206	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor **201** Hiroshi Sugiyama	Signature of Inventor **202** Isao Saito
Hiroshi SUGIYAMA	**Isao SAITO**
Date: July 10, 2001	Date: July 10, 2001
Signature of Inventor **203** Hirokazu Iida	Signature of Inventor **204**
Hirokazu IIDA	
Date: July 10, 2001	Date:
Signature of Inventor **205**	Signature of Inventor **206**
Date:	Date:

SEQUENCE LISTING

<110> SUGIYAMA, Hiroshi
SAITO, Isao
IIDA, Hirokazu

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PHYSIOLOGICALLY ACTIVE PYRROLE IMIDAZOLE DERIVATIVE

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Application deficiencies found during scanning:

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☑ ***Scanned copy is best available.*** Some drawings are too dark.